MIGRATION PATTERNS OF AVIAN EMBRYONIC BONE MARROW CELLS AND THEIR DIFFERENTIATION TO FUNCTIONAL T AND B CELLS

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Previous studies by Moore and Owen (1,2) as well as more recent experiments with interspecific chimeras (3,4) support the concept that blood-borne lymphoid cell precursors immigrate into the bursa as well as into the thymus. These precursors presumably are multipotential stem cells that according to some investigators originate in the yolk sac (5), whereas others propose certain intraembryonic hematopoietic islands as the first recognizable source of stem cells (4,6). By 14-18 days of embryonation, stem cells capable of migrating to the thymus and bursa have been detected in additional hematopoietic sites such as spleen and bone marrow (2). None of the above experiments, however, have formally demonstrated that this migration of cells to the thymus and bursa is in all instances followed by functional differentiation to T and B cells respectively. This is simply assumed and extensively quoted as if it were established fact. It is not without challenge, however, and a recent study concluded that embryonic bone marrow does not harbor bursal precursor cells and suggested that the previously demonstrated migration of 14-18 day embryonic spleen and bone marrow to the bursa may have been of no physiological significance and probably represented nonspecific trapping (7). It is apparent that considerable confusion and controversy exists in relation to early events in T and B cell ontogeny.

We decided to reexamine the question of the presence or absence of precursor cells for thymic and bursal lymphocytes in certain hematopoietic sites at specific stages of embryonic development, to formally establish the capacity of precursor cells to differentiate to T and B cells in their respective microenvironments and to examine whether the thymus and bursa displayed significant differences in regulating the influx of precursor cells and their differ-
entiation at different stages of development. The latter question was prompted by several observations which suggested a definite functional difference between bursa and thymus in this regard. The most important of these was the demonstrated failure of the bursa, in striking contrast to the thymus, to regenerate its lymphoid cell population following either localized irradiation (8) or following repeated administration of cyclophosphamide in the newly hatched (9) as well as in the late embryonic period (10). This could be interpreted as a differential sensitivity of the bursal and thymic microenvironment to these agents, resulting in a selective failure to support cell differentiation in the bursa. The observed difference could, however, also result from 1) a selective failure of precursor cells to gain entrance to the bursal microenvironment beginning in the late embryonic period, 2) the existence of separate populations of precursor T (PT) and precursor B (PB) cells with a selective numerical decrease or disappearance of the latter in the late embryonic or newly hatched period or 3) a combination of these two.

These considerations led to the construction of several models with hypothetical functional differences between thymus and bursa and allowing for multipotential stem cells versus separate lineages of precursor T and precursor B cells respectively. The models could be subjected to experimentation and results obtained analyzed for compatibility with any one model. One model which gained special significance in light of subsequent experimental results is shown in figure 1. This model predicts a migration pattern and differentiation to functional T and B cells following the transfer of precursor cells of a designated developmental stage to recipients of a given age as illustrated. For example, precursor cells of 14 day old embryos transferred to embryos 18 days or older would be expected to differentiate into functional T cells following the migration of PT cells through the thymus. Few or no B cells would develop, however, as the transferred PB cells could no longer gain entrance into the bursa at that stage.

Materials and methods. White Leghorn chick embryos of the SC line (Hy-Line International, Dallas Center, Iowa) homozygous for the major histocompatibility locus, genotype B2B2 were used in all experiments. 30x10^6 viable, sex chromosomally marked bone marrow or spleen cells obtained from embryos or chicks at specific stages of development were injected intravenously into recipients at specific stages of development. Recipient embryos had been exposed 24 hrs previously to 750 R γ-irradiation from a Cs 137 source. At varying intervals after hatching the appropriate chimeras were killed and cell suspensions from the thymus, bursa, bone marrow and spleen prepared for direct chromosome preparations. In addition, aliquots of these cell suspensions were cultured for 48 hours in serum-free medium with a) specific T cell stimulants Concanavalin A (Con A) and Phytohemagglutinin (PHA), b) specific B cell stimulants anti-immunoglobulin (R-antiClg) and dextran sulfate (DxS) and c) appropriate